

In re Patent Application of
Teng Ma
Serial No. 10/645,350
Filed August 21, 2003

In the Specification:

Please substitute the following amended paragraph for text beginning on page 17, line 6, through page 18, line 8. Struckthrough language indicates a deletion, and underlined language indicates an addition. The amendment includes no new matter and is fully supported in the application as filed.

HSC/HPC ex vivo expansion in 3-D perfusion bioreactor apparatus.

Cord blood mononuclear cells were purchased from Clonetics, Walkersville, MD. The bioreactor apparatus is assembled and operated without cells for about ten to twelve hours, although the exact timing may be varied according to need. The bioreactor is then seeded according to the methods described above. Significant benefits in hematopoietic cell output are detectable after 21 days of culture. In a clinical setting, however, longer culture periods may not be suitable waiting periods for patients. Thus, we have examined culture output at days 6, 12, 18 and 24. Human long term culture medium ~~MyeloCult~~ MyeloCult® H5100 (StemCell Technologies Inc., Vancouver, Canada) is used supplemented with serum. Approximately 300 ml of medium is employed for an initial culture period. After three days, about 100 mL of medium is exchanged with new medium and the spent medium is monitored for glucose and lactate concentrations. At days 6, 12, 18, 24, the cells grown in the matrix are sacrificed to determine cell number and cell morphology. The matrices are washed twice with PBS and those cells dislodged will be considered non-adherent cells. The cell-containing matrix is cut into two pieces having approximately equal area. Cell dissociation solution (CDS, ~~Sigma~~ Sigma-Aldrich Co.) is then used to harvest adherent

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cells from the matrix and to minimize alteration of cell surface characteristics. The cellular samples are then evaluated by scanning electronic microscopy (SEM), hematoxylin and eosin (H&E) staining, and immunocytochemistry analysis using confocal laser microscopy (CLSM), flow cytometry, and colony-forming unit assay. Non-adherent and adherent cells will be mixed together for cell counting and assay of colony-forming units. Results from these assays will provide the kinetics of long-term CB expansion (cell counting and CFU assay), metabolic activities (lactate and glucose), cell morphology (Wright's stain and SEM), cell population distribution (flow cytometry), and organization of different types of cells grown in the matrix (immunocytochemistry staining using CLSM). Quantitative results are expressed as the mean \pm SD and statistical analysis was carried out using a commercial software package (~~Minitab~~ Minitab® 11 for Windows, Minitab, Inc., State College, PA).